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Pulse exposure of haematopoietic grafts to prostaglandin E_2 *in vitro* facilitates engraftment and recovery

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Abstract

Objectives: The aim of this study was to evaluate the effects of prostaglandin E_2 (PGE_2) on haematopoietic stem cell (HSC) function and determine its mechanism of action.

Materials and methods: HSC were exposed to PGE_2 for 2 h and effects on their homing, engraftment and self-renewal evaluated *in vivo*. Effects of PGE_2 on HSC cell cycle, CXCR4 expression and migration to SDF-1a were analysed *in vitro*. Apoptosis was evaluated by examination of survivin expression and active caspase-3 levels.

Results: Equivalent haematopoietic reconstitution was demonstrated using 4-fold fewer PGE_2-treated cells compared to controls. Multilineage reconstitution was stable on secondary transplantation, indicating that PGE_2 affects long-term repopulating HSC (LT-HSC) and that enhanced chimaerism of PGE_2-pulsed cells results from their initial treatment. PGE_2 increased CXCR4 expression on mouse and human HSC, increased their migration to SDF-1α *in vitro* and enhanced *in vivo* marrow homing 2-fold, which was blocked by a CXCR4 receptor antagonist. PGE_2 pulse exposure reduced apoptosis of mouse and human HSC, with increase in endogenous caspase inhibitor survivin, and concomitant decrease in active caspase-3. Two-fold more HSC entered the cell cycle and proliferated within 24 h after PGE_2 pulse exposure.

Conclusions: These studies demonstrate that short-term PGE_2 exposure enhances HSC function and supports the concept of utility of PGE_2 as an *ex vivo* strategy to improve function of haematopoietic grafts, particularly those where HSC numbers are limited.

Introduction

Prostaglandins (PGs) are extremely active short-lived lipids that act locally in a paracrine or autocrine fashion. There are no organs, tissues or cells in the body that are not affected by these bioactive lipids, either directly or through accessory mechanisms. All nucleated cells synthesize PGs by cleavage of the essential fatty acid, arachidonic acid from membrane phospholipids by phospholipase A2, oxidation of free arachidonic acid by two cyclooxygenase enzymes (COX1 and COX2) and subsequent isomerization to an intermediate form, and synthesis of various mature PGs by specific synthases (2,3). A schematic outline of prostaglandin E_2 (PGE_2) biosynthesis is shown in Fig. 1. PGE_2 is the

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predominant metabolite of arachidonic acid and is the most abundant eicosanoid (4,5). PGE_2 has been implicated in regulation of numerous physiological systems, and is the primary mediator of symptoms of fever (6) and inflammation (4,7), a regulator of atherosclerosis, blood pressure and strokes (7), and is involved in neoplastic transformation and cancer cell effects on host responses (4,8). Effects of PGE_2 are mediated through four highly conserved G-protein-coupled receptors (EP1–4) with overlapping as well as distinct signalling pathways (9,10) that can lead to seemingly opposing effects. EP receptor levels vary among tissues, and signalling pathways downstream of the same receptors can vary depending upon cell lineage.

Previously we have shown that PGE_2 regulates haematopoiesis, affecting both haematopoietic stem cell (HSC) and progenitor cell (HPC) functions. PGE_2 inhibits myelopoiesis *in vitro* and *in vivo* (11–13), and participates in a negative feedback loop regulating myeloid progenitor cell expansion (12,14,15). In contrast to its effects on myelopoiesis, PGE_2 promotes erythroid and multipotential colony formation *in vitro* (16,17). Early studies showed that short-term *ex vivo* treatment of bone marrow cells with PGE_2 could enhance progenitor cell proliferation (18,19); however, *in vivo* dosing led to little or no effect (20). We later showed that pulse exposure of mouse or human bone marrow cells to PGE_2 stimulated proliferation, cycling and differentiation of quiescent cells leading to an increase in cycling HPC, suggesting that PGE_2 enhances HSC function (21–23). These studies also clearly defined that dose, timing and length of exposure of haematopoietic cells to PGE_2 were critical factors defining stimulatory versus inhibitory effects on haematopoiesis. However, while these studies were the first to clearly implicate PGE_2 in positive regulation of haematopoiesis, stem cell function cannot be defined solely based on clonogenic assays. Elucidation of the effects of PGE_2 on HSC function requires demonstration that transplanted cells can repopulate haematopoiesis in myeloablated hosts (24). Recently, effects of PGE_2 on haematopoietic stem cell function have been revisited and studies in zebrafish and mice (25,26) validate the stimulatory effects of PGE_2 on HSC function that were suggested in earlier *in vitro* studies. In addition, we have defined mechanisms of action of PGE_2 on HSC function, particularly on HSC homing and self-renewal, which suggest that short-term exposure of haematopoietic grafts to PGE_2 can be utilized for therapeutic benefit in HSC transplantation (25). In this study, we describe pre-clinical findings supporting the therapeutic *ex vivo* utility of PGE_2 as a facilitator of HSC engraftment.

Materials and methods

Mouse and human cord blood

C57Bl/6 (CD45.2) mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). B6.SJL-PtrcA-Pep3B/BoyJ (BOYJ) (CD45.1), C57Bl/6 X BOYJ-F1 (CD45.1/CD45.2) and NOD.Cg-*Prkdcscid IL2rgtm1Wjl*/Sz (NSG) mice were bred in-house. Human umbilical cord blood (UCB) was obtained from Wishard Hospital, India-napolis, IN, with IRB approval.

Flow cytometry

For detection of SKL cells, streptavidin conjugated with PE-Cy7 [to stain for biotinylated MACS lineage antibodies (Miltenyi Biotech, Auburn, CA, USA)], c-kit-APC, Sca-1-PE or APC-Cy7, CD45.1-PE, CD45.2-FITC and CD34-PE were used (BD Biosciences, San Jose, CA, USA). For SLAM SKL, we used Sca-1-PE-Cy7, c-kit-FITC, CD150-APC (eBiosciences, San Diego, CA, USA), CD48-biotin (eBiosciences) and streptavidin-PE. UCB $CD34^+$ cells were detected using anti-human-CD34-APC. For multi-lineage analysis, APC-Cy7-Mac-1, PE-Cy7-B-220 and APC-CD3 were used (BD Biosciences).

PGE_2 pulse exposure

Cells were incubated with 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$) (Cayman Chemicals, Ann Arbor, MI, USA) (reconstituted in 100% ethanol) diluted in media, on ice, for 2 h. After incubation, cells were washed twice before use. Vehicle-treated cells were pulsed with equivalent concentration of ethanol.

Limiting dilution competitive and non-competitive transplantation

Whole bone marrow cells (CD45.2) were treated with 1 μM $dmPGE_2$ or 0.001% EtOH, washed, admixed with 2×10^5 congenic CD45.1 competitor marrow cells at various ratios, and transplanted into lethally irradiated CD45.1/CD45.2 mice. Peripheral blood CD45.1 and CD45.2 cells were determined monthly by FACS. HSC frequency was quantified by Poisson statistics using L-CALC software (Stem Cell Technologies, Vancouver BC, Canada). Competitive repopulating units (CRU) were calculated as described by Harrison (24). For secondary transplantation, 2×10^6 WBM from previously transplanted CD45.1/CD45.2 mice at 20 weeks post-transplant were injected into lethally irradiated CD45.1/CD45.2 mice in a non-competitive fashion.

HSPC homing

For competitive homing studies, SKL cells from CD45.2 and CD45.1 mice were FACS sorted, treated with $dmPGE_2$ or vehicle and 3×10^4 CD45.1 (vehicle or $dmPGE_2$ treated) plus 3×10^4 CD45.2 ($dmPGE_2$ or vehicle treated) SKL cells transplanted into lethally irradiated CD45.1/CD45.2 mice. Homed CD45.1 and CD45.2 SKL cells in bone marrow were determined after 16 h. To evaluate the role of CXCR4 in homing, Lin^{neg} CD45.2 cells were treated with vehicle or 1 μM $dmPGE_2$ plus 10 μM AMD3100 (AnorMed Inc., Vancouver, BC, Canada), and 2×10^6 treated cells injected into lethally irradiated CD45.1 mice and homed SKL cells analysed 16 h post-transplant. Homing of human $CD34^+$ cells was evaluated in NSG mice. UCB mononuclear cells were pulsed with $dmPGE_2$ or vehicle and 4×10^7 cells transplanted into each of five sublethally irradiated (250 cGy) NSG mice. Homed $CD34^+$ cells were analysed 16 h post-transplantation.

Analysis of CXCR4 expression and apoptosis

Mouse SKL cells or $CD34^+$ UCB cells were pulsed with $dmPGE_2$ or vehicle, washed and cultured in RPMI-1640/10% HI-FBS at 37 °C for 24 h. CXCR4 expression was analysed using CXCR4-PE. Apoptotic levels were measured with FITC-annexin-V or FITC-anti-active caspase-3 after culture in RPMI-1640/2% HI-FBS at 37 °C for 24 h. For survivin and

active caspase-3 detection, cells were permeabilized and fixed using the CytoFix/CytoPerm kit (BD Biosciences) and stained with anti-active caspase-3-FITC Flow Kit (BD Biosciences) or survivin-PE (R&D Systems, Minneapolis, MN, USA).

Survivin and active caspase-3

Mouse SKL cells or $CD34^+$ UCB cells were pulsed with $dmPGE_2$ or vehicle, washed and cultured in RPMI-1640/10% HI-FBS at 37 °C for 24 h. CXCR4, survivin and/or active caspase-3 were analysed by FACS.

Migration assays

Chemotaxis to SDF-1a *in vitro* was determined as described previously (27). Briefly, $dmPGE_2$-and vehicle-treated $Lineage^{neg}$ bone marrow cells, UCB $CD34^+$ cells or peripheral blood $CD34^+$ cells mobilized by G-CSF were cultured in RPMI/10% HI-FBS overnight, washed, 2×10^5 cells added to the top chamber of transwells, with or without rmSDF-1α (R&D Systems) in the bottom chamber, and incubated for 4 h at 37 °C. Cells migrating to the bottom chamber were enumerated by FACS. Per cent migration was calculated by dividing total cells migrated to lower well, by cell input, multiplied by 100.

Cell cycle analysis

$Lineage^{neg}$ cells were treated with $dmPGE_2$ or vehicle and cultured with rmSCF (50 ng/ml) (R&D Systems), rhFlt-3 and rhTPO (100 ng/ml each) (Immunex, Seattle, WA, USA) for 20 h. Cells were stained for SLAM SKL, fixed, permeabilized and stained with Hoechst-33342 followed by Pyronin-Y. Proportion of SLAM SKL cells in G_0, G_1, S and G_2/M phase was determined by FACS.

Statistical analysis

All pooled values are expressed as mean ± SEM. Statistical differences were determined using paired or unpaired two-tailed *t*-test functions with Microsoft Excel as appropriate.

Results

PGE_2 increased long-term stem cell engraftment

Competitive repopulation transplantation models in mice allow for quantification of the ability of HSC to repopulate haematopoiesis. When congenic CD45.1 and CD45.2 mouse marrow cells were pulsed with vehicle or PGE_2 and transplanted in a limiting-dilution fashion, into CD45.1/CD45.2 hybrid mice, a model that permits head-to-head comparison of HSC populations; significantly enhanced HSC engraftment was observed for PGE_2 -pulsed bone marrow, compared to controls (Table 1). At 5 months post-transplantation, analysis of peripheral blood chimaerism showed significantly higher levels of white blood cells derived from PGE_2-treated marrow cells. Analysis of HSC frequency by Poisson statistics or by calculation of competitive repopulating units (CRU), indicated an ~4-fold increase in HSC frequency, strongly suggesting a direct effect on HSC. These results agree with similar studies performed in a conventional congenic transplant model (26). Myeloid cells, B and T lymphocytes were reconstituted with no obvious bias in lineage

reconstitution. To validate self-renewal capacity of *ex vivo* PGE_2-treated repopulating cells, marrow from primary transplantation recipients was harvested and transplanted into secondary recipients without any further *ex vivo* manipulation. Analysis of chimaerism at 24 weeks after secondary transplantation also showed significantly increased contribution to chimaerism from PGE_2-treated cells from primary recipients (Table 1). That enhanced chimaerism resulting from PGE_2 exposure in primary donors was maintained in secondary transplants without additional treatment, indicated that the effect of PGE_2 pulse exposure was stable and manifest in long-term repopulating stem cells (LT-HSC). On further serial trans-plantation, persistent enhanced chimaerism resulting from PGE_2 pulse exposure prior to transplantation into primary donors, was observed. Transplantation of human haematopoietic cells in immunodeficient mice offers a model system to evaluate human HSC function *in vivo* (28). In a similar fashion to that shown using mouse bone marrow cells, short-term *ex vivo* pulse exposure to PGE_2 enhanced engraftment of human UCB HSC in NOD/SCID-IL2-γ-receptor null (NSG) mice (Table 1).

PGE_2 increased HSC homing efficiency

Effects on cell division, homing or proliferation can positively or negatively affect HSC function. As we have previously shown that PGE_2 can have dual effects on haematopoiesis, we sought to define its mechanism of action to better understand the potential clinical utility of transient *ex vivo* PGE_2 exposure. Successful haematopoietic reconstitution requires that administered haematopoietic cells traffic/home to bone marrow niches where they can engraft, self-renew and differentiate. We therefore evaluated effects of PGE_2 exposure on these HSC functions.

To evaluate homing efficiency of HSC and determine whether the effect of PGE_2 pulse exposure results from direct PGE_2 effect, or is mediated through accessory cells, we FACS sorted congenic mouse $Lineage^{neg}$ $Sca\text{-}1^+$, $c\text{-}kit^+$ (SKL) cells, enriched for LT-HSC, treated them *ex vivo* and directly compared homing efficiency of PGE_2-treated and vehicle-treated SKL cells head-to-head in CD45 hybrid mice. Pulse exposure of purified SKL cells to PGE_2 increased their homing efficiency 2-fold compared to controls (Fig. 2a). In traditional homing assays, increased homing of more differentiated $Lineage^{neg}$, $c\text{-}kit^+$ HPC and $Lineage^{neg}$ cells was not observed, suggesting that the enhanced homing effect of PGE_2 is specific to HSC.

The CXCR4 and SDF-1a axis is a critical component of HSC marrow homing (29), and overexpression of CXCR4 in HSC enhances their *in vivo* homing and engraftment (30–32). To determine whether up-regulated CXCR4 would mediate PGE_2-enhanced HSC marrow homing, marrow cells were treated *ex vivo* with selective CXCR4 antagonist AMD3100 (33) after PGE_2 pulse exposure and prior to transplantation (Fig. 2b). As expected, PGE_2 pulse exposure increased SKL cell homing. Incubation of vehicle or PGE_2-pulsed cells with AMD3100 reduced SKL cell homing and abrogated PGE_2 -improved homing efficiency. Reduction in HSC homing by AMD3100 is consistent with previous reports (34,35). To verify the enhancing effect of PGE_2 on human cells, $CD34^+$ cells were isolated from cord blood, pulsed with PGE_2 or vehicle and their homing capacity analysed in NSG mice, a

validated model for human HSC homing (36). PGE_2 pulse exposure significantly enhanced homing efficiency of human UCB $CD34^+$ cells in NSG mice (Fig. 2c).

PGE_2 increased HSC CXCR4 and migration to SDF-1α

As PGE_2 enhancement of HSC homing was blocked by CXCR4 antagonism and PGE_2 has been reported to upregulate CXCR4 on $CD34^+$ cells (37) and endothelial cells (38), and increase monocyte chemotaxis to SDF-1α (39), we evaluated whether improved homing would result from HSC up-regulation of SDF-1α/CXCR4 signalling and function. Pulse exposure to PGE_2 increased CXCR4 expression on mouse SKL cells and human UCB $CD34^+$ cells (Table 2). HSC selectively migrate to SDF-1α *in vitro* (40), a process that reflects their *in vivo* homing capacity. Vehicle-and PGE_2-treated mouse SKL cells and $CD34^+$ cells from human UCB and G-CSF mobilized peripheral blood all demonstrated significant migration to 100 ng/ml SDF-1α; however, in all cases, chemotaxis of PGE_2-treated cells was significantly higher (Table 2), indicating that up-regulation of HSC CXCR4 coincided with enhanced migratory funct ion. The enhancing effect of PGE_2 on chemotaxis to SDF-1α was blocked by AMD3100, indicating that the observed effect was mediated through the CXCR4 receptor.

PGE_2 decreased apoptosis

Apoptosis is an important regulatory process in normal and malignant haematopoiesis and signalling downstream of PGE_2 receptors has been implicated in anti-apoptotic effects (41–43). This suggests that enhancement of HSC function by PGE_2 could result from enhanced HSC survival. Under reduced serum conditions, pulse exposure to PGE_2 reduced annexin-V and active caspase-3 in SKL cells, markers of apoptosis in mouse and human HSC (Fig. 3a,b). Furthermore, intracellular levels of the endogenous caspase-3 inhibitor survivin, were significantly higher in both SKL and $CD34^+$ cells, consistent with our previous findings that survivin regulated apoptosis and proliferation in HSC (44,45), and studies by others that PGE_2 can increase survivin levels in cancer cells (46,47). QRT-PCR analysis of treated SKL cells and UCB $CD34^+$ cells showed similarly elevated levels of survivin mRNA.

PGE_2 increased entry of HSC into the cell cycle

We have previously shown that survivin regulates entry of HSC into the cell cycle and their progression through it (44,45). Furthermore, β-catenin, which is implicated in HSC proliferation/self-renewal, lies downstream of EP receptor pathways (48). Ability of PGE_2 to modulate these cell cycle regulators suggests that increase in HSC self-renewal and proliferation might contribute to enhanced engraftment observed using PGE_2-pulsed cells. PGE_2 pulse exposure significantly increased the proportion of primitive LT-HSC, defined as SLAM ($CD150^+$, $CD48^-$) SKL cells, in the cell cycle (G_1 + S/G_2M) compared to controls (Fig. 4). No significant effect on rate of cell cycle completion by HPC or more differentiated cells, was observed (data not shown), strongly suggesting that effects of PGE_2 on cell cycle rate were selective.

Discussion

In vivo analysis of effects of short-term pulse exposure to PGE_2 clearly demonstrated that it has direct and stable effects on HSC function. Enhancement of HSC frequency and engraftment by PGE_2 treatment results from effects on HSC homing and cell cycle activity involving up-regulation of CXCR4 and survivin, with increased chemotactic response to SDF-1α and reduced apoptosis. Ability to facilitate homing, survival and proliferation of HSC by short-term *ex vivo* PGE_2 exposure offers an exciting clinical translation strategy to improve haematopoietic transplantation, specially in transplant settings characterized by low HSC numbers, such as with umbilical cord blood cells and some mobilized peripheral blood stem cell products. Our experimental pre-clinical limiting dilution transplantation studies show that equivalent engraftment is achieved with 4-fold fewer PGE_2-treated cells compared to untreated cells. Homing and migration studies utilizing UCB $CD34^+$ cells also clearly support potential translation of short-term PGE_2 exposure to human haematopoietic grafts.

While *ex vivo* utility of PGE_2 is clear, it will be interesting to determine whether enhanced HSC engraftment/recovery could also be achieved by administering PGE_2 *in vivo* or if PGE_2 used *in vivo* could further facilitate engraftment of HSC exposed to PGE_2 *ex vivo*. In COX2 knockout mice, haematopoietic recovery from chemotherapy is delayed (49) suggesting that PGE_2 production is critical for HSC expansion. However, it must be kept in mind that while short-term exposure to PGE_2 can enhance HSC function, prolonged administration inhibits myelopoiesis (11,50), which is supported by inhibition of prostaglandin biosynthesis *in vivo* leading to expansion of myelopoiesis (51), at least in mice. Nevertheless, combination of transient *ex vivo* PGE_2 exposure with selective modulation of *in vivo* PGE_2 biosynthesis may result in significant further improvements in HSC function.

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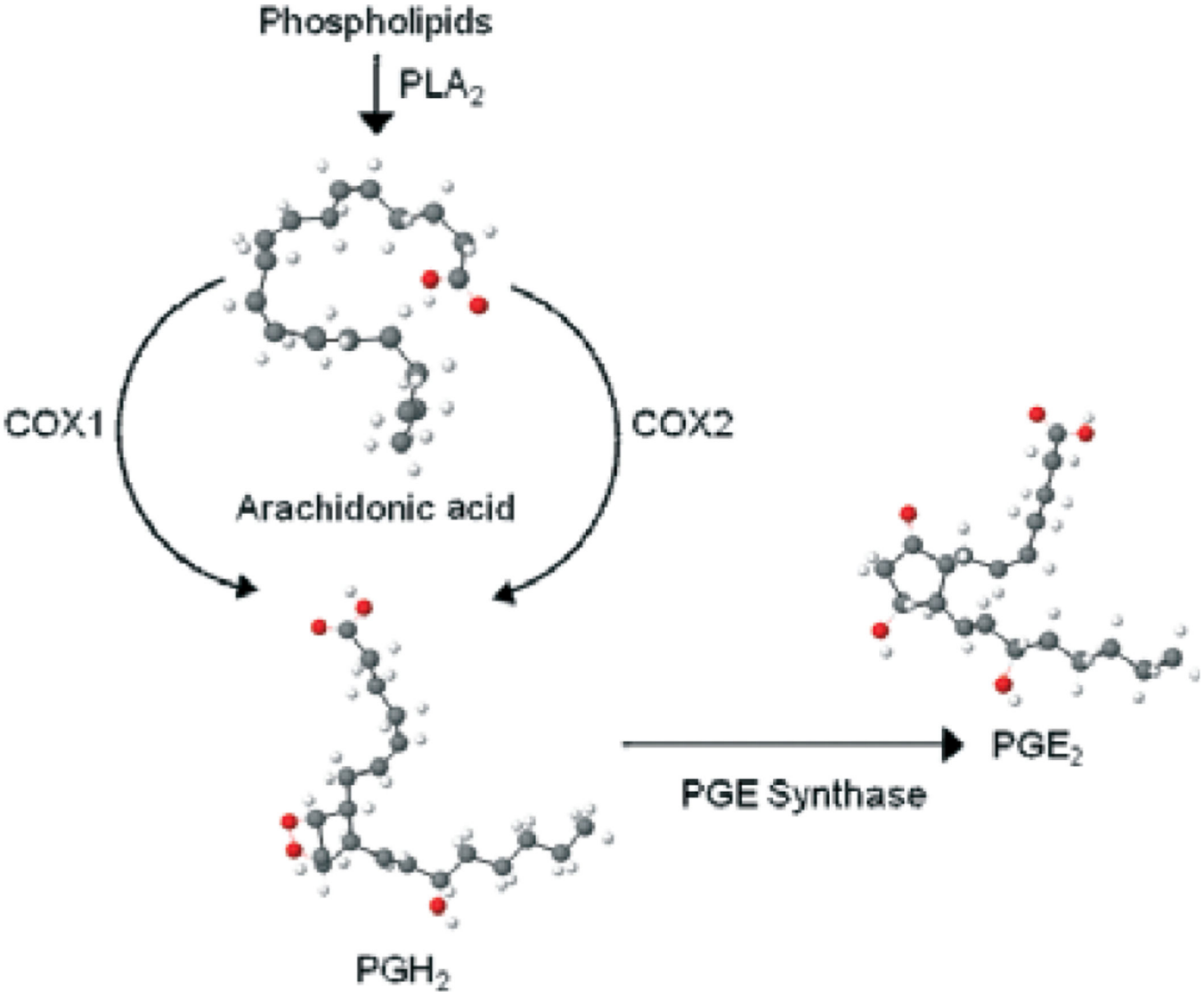

Figure 1. Outline of prostaglandin E_2 (PGE_2) biosynthesis from membrane phospholipids.
COX, cyclooxygenase; PLA_2, phospholipase A_2.

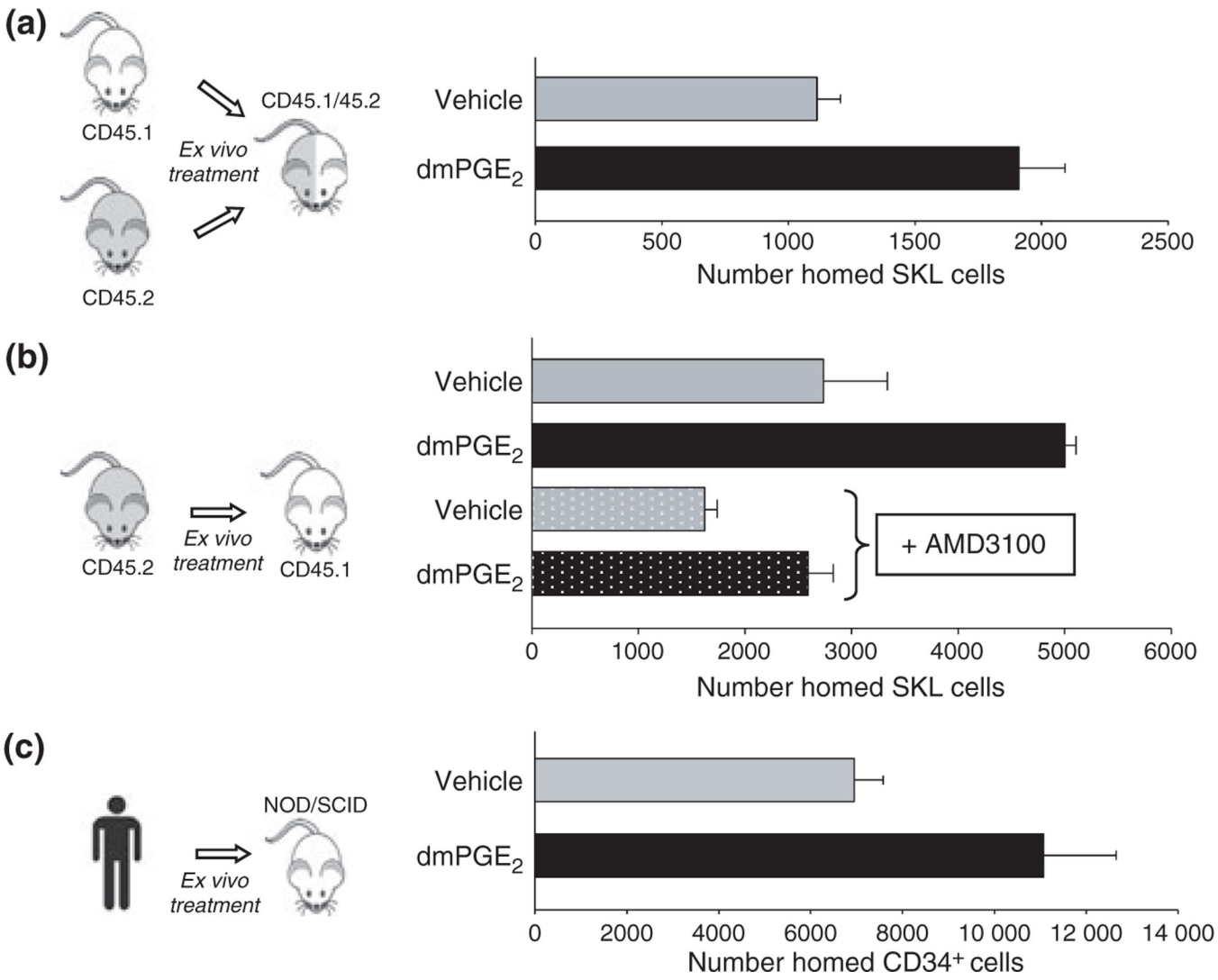

Figure 2. PGE_2 pulse exposure enhances homing of mouse and human HSC.

(a) SKL cells were isolated from congenic mice by FACS sorting and treated with vehicle or 1 μM 16, 16-dimethyl PGE_2 ($dmPGE_2$) on ice for 2 h. After incubation, cells were washed and 3×10^4 vehicle-or $dmPGE_2$-treated CD45.1 and 3×10^4 vehicle-or $dmPGE_2$-treated CD45.2 cells injected into lethally irradiated CD45.1/CD45.2 hybrid mice. Number of homed CD45.1 and CD45.2 SKL cells was quantified in bone marrow at 16 h post-transplantation. Data (mean ± SEM) from 10 mice with treatment groups of five mice, switched to rule out bias. (b) Bone marrow cells from CD45.2 mice were treated with vehicle or $dmPGE_2$, washed, incubated in the presence or absence of 10 μM AMD3100 for 30 min and transplanted into lethally irradiated CD45.1 mice. Number of homed CD45.2 SKL cells was quantified in bone marrow at 16 h post-transplantation. Data (mean ± SEM) from three mice per group each assayed individually. (c) Umbilical cord blood mononuclear cells were isolated, treated with vehicle or $dmPGE_2$ and injected into sublethally irradiated NSG mice. Bone marrow was analysed after 16 h for number of homed human $CD45^+$, $CD34^+$ cells. Data (mean ± SEM) from five mice per group each assayed individually.

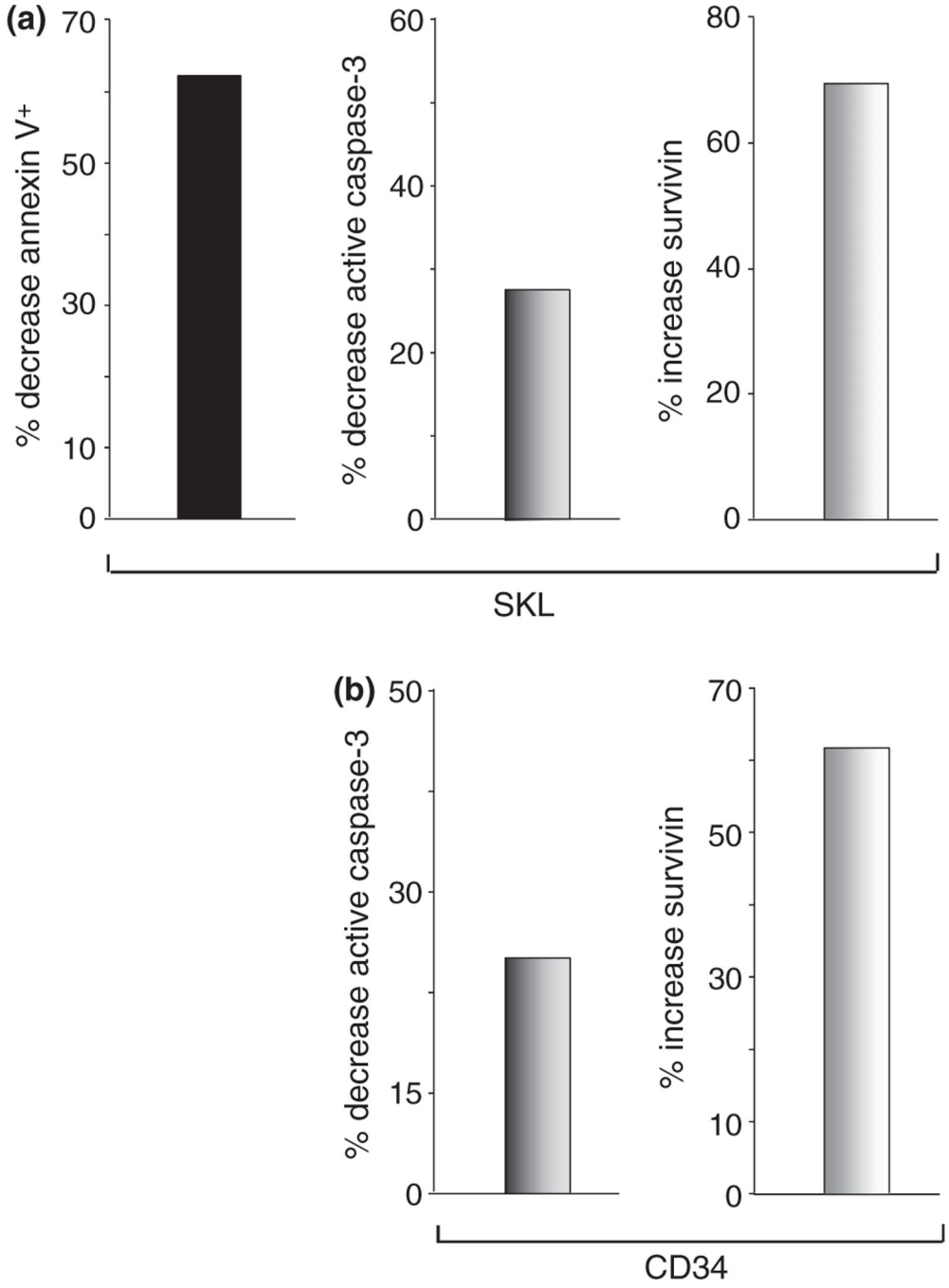

Figure 3. PGE_2 decreases apoptosis and increases level of intracellular survivin.

(a) Lineage-depleted mouse bone marrow cells were treated with $dmPGE_2$ or vehicle for 2 h, washed and cultured in 2% HI-FBS without growth factors for 24 h at 37 °C. After culture, cells were stained for SKL. Apoptosis was measured with FITC-annexin-V (left panel). Replicate cells were permeabilized and stained with anti-active caspase-3-FITC (middle panel) or anti-survivin-PE (right panel). Data expressed as fold-change induced by $dmPGE_2$ pulse exposure compared to vehicle-treated cells. (b) Fold-change in active caspase-3 (left panel) and intracellular survivin (right panel) in permeabilized PGE_2-treated UCB $CD34^+$

cells relative to vehicle-treated cells after culture in 2% HI-FBS without growth factors for 24 h at 37 °C.

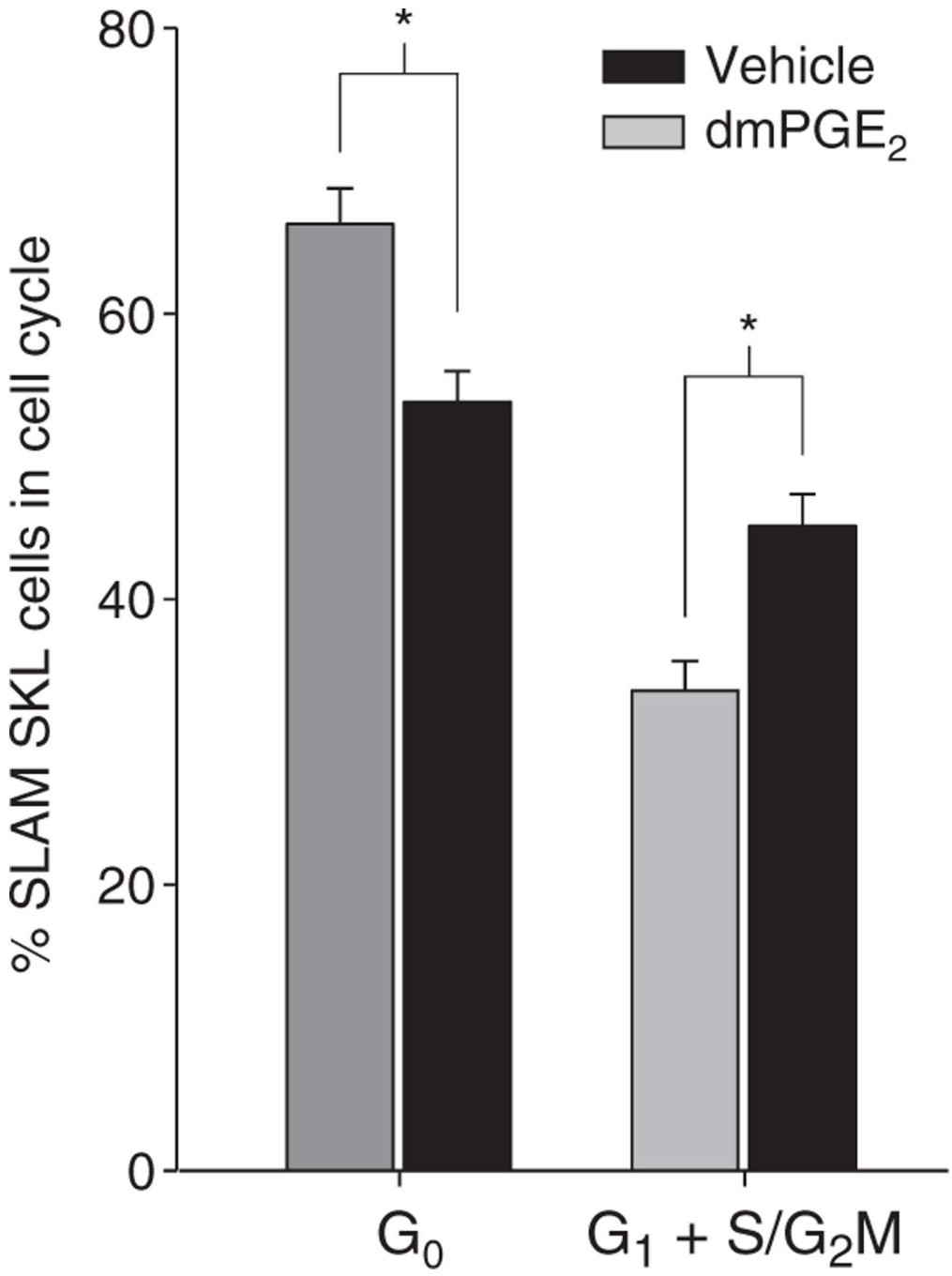

Figure 4. PGE_2 increases cell cycle of SLAM SKL cells.

Lineage-depleted mouse bone marrow cells were treated with $dmPGE_2$ or vehicle for 2 h, washed and cultured with 50 ng/ml rmSCF and 100 ng/ml each of rhFlt3 and rhTpo for 20 h. Cells were stained for SLAM ($CD150^+$, $CD48^-$)) SKL, Hoechst-33342 and pyronin-Y and proportion of SLAM SKL cells in G_0, G_1, S and G_2/M phases of the cell cycle determined by flow cytometry. Data (mean ± SEM) from nine mice each assayed individually. $*P < 0.05$.

Table 1.

Transient exposure of haematopoietic cells to PGE_2 enhances HSC engraftment

Group	Time of exposure (h)[a]	Fold increase HSC frequency[b]	Fold increase CRU[c]	% PB chimaerism congenic mice[d] Primary	% PB chimaerism congenic mice[d] Secondary	% PB chimaerism NSG[e]
Vehicle	2	–	–	13.3 ± 1.4	5.1 ± 0.13	2.3
1 μM $dmPGE_2$	2	4.12	3.4	40.7 ± 5.0 *	34.2 ± 0.51 *	11.0

[a] Whole mouse bone marrow cells or human umbilical cord blood mononuclear cells were treated on ice with 0.001% ethanol or 1 μM $dmPGE_2$ for 2 h and washed twice in ice-cold PBS before transplant.

[b] HSC cell frequency was calculated by Poisson statistics at 20 weeks post-transplant (P_0 vehicle = 89 586; P_0 PGE_2 = 21 753) using L-CALC software (Stem Cell Technologies, Vancouver, BC, Canada).

[c] CRU were calculated based on PB chimaerism at 20 weeks in primary transplants according to the method of Harrison (24).

[d] Chimaerism in PB is shown at 20 weeks in primary transplant and 24 weeks in secondary transplant (mean ± SEM). Data are for 10 mice in two experiments, each assayed individually.

[e] Chimaerism in PB of NSG (*NOD.Cg-Prkdcscid IL2rgtm1 Wjl/ Sz*) mice at 8 weeks post-transplant with vehicle-or PGE_2-pulsed umbilical cord blood mononuclear cells.

* $P < 0.05$.

Table 2.

PGE_2 enhances mouse and human HSC CXCR4 expression and migration to SDF-1α

	SKL cells		UCB $CD34^+$ cells[a]		MPB $CD34^+$ cells[a]	
	Vehicle	$dmPGE_2$[b]	Vehicle	$dmPGE_2$[b]	Vehicle	$dmPGE_2$[b]
% increase CXCR4[c]	6.99 ± 2.5	26.82 ± 4.4*	6.88	17.31	–	–
% migration to SDF-1[d]	46.8 ± 4.8	67.1 ± 5.7*	27.7 ± 1.2	55.6 ± 3.3*	23.2 ± 1.1	41.4 ± 1.8*

[a] $CD34^+$ cells were isolated from umbilical cord blood or apheresis products of normal donors mobilized with G-CSF for allogeneic transplantation.

[b] Mouse SKL cells or human $CD34^+$ cells were treated on ice with 0.001% ethanol or 1 μM $dmPGE_2$ for 2 h, washed, and resuspended in media with 10% HI-FCS and cultured at 37 °C for 16 h.

[c] Cells were pulsed with $dmPGE_2$ and cultured as described above. CXCR4 expression was analysed by flow cytometry. Data are expressed as percent-age change in mean fluorescence intensity of CXCR4 expression resulting from treatment with vehicle or $dmPGE_2$.

[d] Cells were pulsed with $dmPGE_2$ and cultured as described above. After incubation, cells were washed, resuspended in RPMI/0.5% BSA and allowed to migrate to 100 ng/ml recombinant mouse or human SDF-1a for 4 h. Total cell migration was quantified by flow cytometry. Data (mean ± SEM) are from three experiments.

* $P < 0.05$.